

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising a nucleotide sequence that encodes a polypeptide comprising an ExtN, a ExtC, and an Int  
5 interposed between said ExtN and said ExtC, wherein:  
    said ExtN is the N-terminal portion of the polypeptide;  
    said Int is an intein; and  
    said ExtC is the C-terminal portion of the polypeptide,  
    and wherein at least a portion of said nucleotide sequence has  
10 been modified to contain plant optimized codons.
2. An isolated polynucleotide comprising a nucleotide sequence that encodes a fusion polypeptide consisting of an ExtN, a ExtC, and an Int interposed between said ExtN and said ExtC, wherein:  
    said ExtN is the N-terminal portion of the polypeptide;  
15      said Int is an intein; and  
    said ExtC is the C-terminal portion of the polypeptide.
3. The polynucleotide of Claim 1 or 2 wherein said Int is of bacterial origin.
4. The polynucleotide of Claim 1 or 2 that further comprises a  
20 regulatory sequence.
5. The polynucleotide of Claim 4 wherein said regulatory sequence is selected from the group consisting of a constitutive plant promoter, a plant tissue-specific promoter, and a plant developmental stage-specific promoter.
- 25 6. The polynucleotide of Claim 1 or 2 wherein said Int is a naturally split intein consisting of an IntN and an IntC, wherein:  
    said IntN is the N-terminal portion of said naturally split intein; and  
    said IntC is the C-terminal portion of said naturally split  
30 intein.
7. The polynucleotide of Claim 6 wherein said nucleotide sequence comprises:  
    an N-nucleotide sequence encoding said ExtN and said IntN;  
    and  
35      a C-nucleotide sequence encoding said IntC and said ExtC.
8. The polynucleotide of Claim 7 that further comprises an N-regulatory sequence that is operably linked to said N-nucleotide sequence and a C-regulatory sequence that is operably linked to said

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C-nucleotide sequence, and wherein said C-regulatory sequence is interposed between said N-nucleotide sequence and said C-nucleotide sequence.

5        9. The polynucleotide of Claim 6 wherein said IntN is encoded by the nucleotide sequence of SEQ ID NO:22.

10. The polynucleotide of Claim 6 wherein said IntC is encoded by the nucleotide sequence of SEQ ID NO:24.

11. The polynucleotide of Claim 6 wherein said IntN has the amino acid sequence of SEQ ID NO:23.

10        12. The polynucleotide of Claim 6 wherein said IntC has the amino acid sequence of SEQ ID NO:25.

13. The polynucleotide of Claim 1 or 2 wherein said ExtN and said ExtC together form an active protein.

14. A vector comprising the polynucleotide of Claim 1 or 2.

15        15. A host cell comprising the polynucleotide of Claim 1 or 2.

16. A transgenic plant comprising the polynucleotide of Claim 1 or 2.

17. A seed comprising the polynucleotide of Claim 1 or 2.

18. An isolated polynucleotide comprising a nucleotide sequence that encodes a polypeptide selected from the group consisting of:  
an ExtN and an IntN; and  
an ExtC and an IntC,  
wherein said IntN and said IntC together form a naturally split intein.

25        19. A vector comprising the polynucleotide of Claim 18.

20. A host cell comprising the polynucleotide of Claim 18.

21. A transgenic plant comprising the polynucleotide of Claim 18.

22. A seed comprising the polynucleotide of Claim 18.

23. A method for producing a protein comprising an ExtN and a  
30        ExtC, said method comprising:

(a) obtaining an N-nucleotide sequence that encodes an N-polypeptide comprising an ExtN and an IntN;

(b) obtaining a C-nucleotide sequence that encodes a C-polypeptide comprising an IntC and an ExtC;

35        (c) transforming a plant host with said N-nucleotide sequence and said C-nucleotide sequence such that said plant produces said protein; and

(d) optionally recovering said protein.

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24. The method of Claim 23 wherein said (c) transforming comprises transforming said plant host with a vector that comprises said N-nucleotide sequence and said C-nucleotide sequence.

5 25. The method of Claim 23 wherein said (c) transforming comprises separately transforming said plant host with said N-nucleotide sequence and said C-nucleotide sequence.

26. The method of Claim 23 wherein at least a portion of at least one of said N-nucleotide sequence and said C-nucleotide sequence has been modified to contain plant optimized codons.

10 27. The method of Claim 23 wherein said IntN and said IntC together form a naturally split intein.

28. The method of Claim 23 wherein said IntN and said IntC together form an intein of bacterial origin.

15 29. The method of Claim 23 wherein said plant host is a plant, a plant derived tissue, or a plant cell.

30. The method of Claim 23 wherein said plant host is selected from food plants, non-food plants, arboreous plants, and aquatic plants.

31. The method of Claim 23 wherein said protein consists of said ExtN and said ExtC.

20 32. The method of Claim 31 wherein said protein is an active protein.

33. A method for producing a protein that comprises an ExtN and a ExtC, said method comprising:

- 25 (a) transforming an N-plant host with an N-polynucleotide comprising an N-nucleotide sequence that encodes an N-polypeptide comprising said ExtN and an IntN, such that said N-plant host produces said N-polypeptide;
- 30 (b) transforming a C-plant host with a C-polynucleotide comprising a C-nucleotide sequence that encodes a C-polypeptide comprising a IntC and said ExtC, such that said C-plant host produces said C-polypeptide; and
- (c) crossing said N-plant host and said C-plant host to obtain a progeny of said N-plant host and said C-plant host, wherein said progeny comprises said protein.

35 34. The method of Claim 33 wherein at least a portion of at least one of said N-nucleotide sequence and said C-nucleotide sequence has been modified to contain plant optimized codons.

35. The method of Claim 33 wherein said IntN and said IntC form a

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naturally split intein.

36. The method of Claim 33 wherein said IntN and said IntC together form an intein that is of bacterial origin.

37. The method of Claim 33 wherein each of said N-plant host and  
5 said C-plant host is a plant, a plant derived tissue, or a plant cell.

38. The method of Claim 33 wherein said plant host is selected from food plants, non-food plants, arboreous plants, and aquatic plants.

39. The method of Claim 33 wherein said (a) transforming comprises introducing an N-vector into said N-plant host and wherein said  
10 N-vector comprises said N-nucleotide sequence, and wherein said (b) transforming comprises introducing a C-vector into said C-plant host and wherein said C-vector comprises said C-nucleotide sequence.

40. The method of Claim 33 wherein said protein consists of said ExtN and said ExtC.

41. The method of Claim 40 wherein said protein is an active  
15 protein.

42. A method for producing a protein comprising an ExtN and a ExtC, said method comprising:

- 20 (a) transforming an N-plant host with an N-polynucleotide comprising an N-nucleotide sequence that encodes an N-polypeptide comprising said ExtN and an IntN, such that said N-plant host produces said N-polypeptide;
- (b) transforming a C-plant host with a C-polynucleotide comprising a C-nucleotide sequence that encodes a  
25 C-polypeptide comprising a IntC and said ExtC, such that said C-plant host produces said C-polypeptide;
- (c) isolating said N-polypeptide from said N-plant host and said C-polypeptide from said C-plant host; and
- 30 (d) combining said N-polypeptide and said C-polypeptide *in vitro* to obtain said protein.

43. The method of Claim 42 wherein at least a portion of at least one of said N-nucleotide sequence and said C-nucleotide sequence has been modified to contain plant optimized codons.

44. The method of Claim 42 wherein said IntN and said IntC  
35 together form a naturally split intein.

45. The method of Claim 42 wherein said IntN and said IntC together form an intein that is of bacterial origin.

46. The method of Claim 42 wherein each of said N-plant host and

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said C-plant host is a plant, a plant derived tissue, or a plant cell.

47. The method of Claim 42 wherein said plant host is selected from food plants, non-food plants, arboreous plants, and aquatic plants.

48. The method of Claim 42 wherein said (a) transforming  
5 comprises introducing an N-vector into said N-plant host and wherein said N-vector comprises said N-nucleotide sequence, and wherein said (b) transforming comprises introducing a C-vector into said C-plant host, said C-vector comprising said C-nucleotide sequence.

49. The method of Claim 48 wherein said protein consists of said  
10 ExtN and said ExtC.

50. The method of Claim 49 wherein protein is an active protein.

51. A transgenic plant that produces an active protein comprising an ExtN and a ExtC, wherein said protein is produced from a polynucleotide comprising a nucleotide sequence that encodes said ExtN,  
15 said ExtC, and an intein interposed between said ExtN and said ExtC.

52. The plant of Claim 51 wherein at least a portion of said nucleotide sequence has been modified to contain plant optimized codons.

53. The plant of Claim 51 wherein said protein is expressed in at least one of a leaf, a root, a stem, a flower, a fruit, or a seed of the plant.

20 54. The plant of Claim 51 that is selected from food plants, non-food plants, arboreous plants, and aquatic plants.

55. A transgenic plant that expresses a polypeptide selected from the group consisting of:

25 an ExtN and an IntN; and  
an ExtC and an IntC,  
wherein said IntN and said IntC together form an intein, and  
wherein said ExtN and said ExtC together form an active  
protein.

30 56. The plant of Claim 55 wherein said polypeptide is expressed in at least one of a leaf, a root, a stem, a flower, a fruit, or a seed of the plant.

57. The plant of Claim 55 that is selected from food plants, non-food plants, arboreous plants, and aquatic plants.